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A METHOD OF TESTING FOR HEPATIC CIRRHOSIS USING A BREATH ANALYSIS APPARATUS AND THE APPARATUS

TECHNICAL FIELD

The present invention relates to a method of testing for hepatic diseases and a breath analyzing apparatus used for the method.

BACKGROUND ART

Diagnosis of hepatic diseases is performed by a clinical view by a doctor, a liver biopsy, a ventroscopy, liver scanning, a ultrasonic examination, CT scanning, X-ray inspection, or the like.

However, since these methods need a special technique by a doctor, a special engineer, or the like, and expensive equipment, they are not suitable for the purpose of testing for hepatic diseases in a general medical checkup or the like.

For this reason, in a medical checkup, the hepatic diseases is examined by extracting blood and urine and analyzing metabolites in the blood and the urine.

As examination of such hepatic diseases, there are methods of measuring of blood serum bilirubin, ZTT, TTT, ALP, CHE, GOT and GPT, gamma-GTP, LDH, LAP, blood serum total protein, a A/G ratio, urine bilirubin, urine urobilinogen, or the like. When it is indicated in such test that the subject may suffer from hepatic diseases, he undergoes further examination and analysis as mentioned above in a medical institution. On the other hand, in recent years, there is proposed a method of testing for various disorders by measuring metabolites in breath. Such a method is described,

for example in Yasuhiro Mitsui, "detection system of trace components in breath", bulletin of S14-5 Showa 62 National Convention of Institute of Electrical Engineers of Japan (1987)).

However, a test for hepatic diseases by measuring metabolites in blood and urine has a disadvantages that it takes much time to obtain a result. Therefore, it may be suitable for neither monitoring of a patient in a medical institution, nor testing in the case of urgent hospitalization. Moreover, blood collecting can be conducted only by a person having a certain qualification, and furthermore, it inflicts a pain on the patient, which causes a problem especially if the patient is in a serious condition or a child. Furthermore, the above-mentioned measurement data of metabolites in blood and urine is not always specific for hepatic diseases. Therefore, in order to perform the most exact diagnosis, it is important to obtain as many measurement data as possible and to make diagnosis with a combination of those data. There has not been disclosed a specific method of using the above-mentioned breath analysis for testing for hepatic diseases.

An object of the present invention is to provide a method of testing for hepatic diseases which enables a quick judgment, inflicts little pain on a patient, and can provide an exact judgment, and an apparatus used for the method.

DISCLOSURE OF THE INVENTION

In order to achieve the above-mentioned object, the inventors of the present invention have aimed at breath analysis which inflicts almost no pain on a patient, and have studied thoroughly about the relation between components in

breath and hepatic diseases, and thereby have completed the present invention.

(1) The present invention relates to a method of testing for hepatic diseases comprising collecting breath, quantifying isopropanol and/or cyanides in the breath, and analyzing a result thereof.

(2) The present invention also relates to the above testing method for testing for hepatic cirrhosis.

Furthermore, the present invention relates to the following breath analyzing apparatuses for testing hepatic diseases.

(3) A breath analyzing apparatus for testing hepatic diseases comprising a breath collecting section for introducing breath to be analyzed, a breath analyzing section wherein isopropanol and/or cyanides in the breath are quantified, and a data-processing section which analyzes the measured result obtained by the breath analyzing section.

(4) The breath analyzing apparatus described in (3) wherein the breath collecting section consists of a breath collecting means and a breath transfer means.

(5) The breath analyzing apparatus described in (4) wherein the breath collecting means is a mouthpiece or a mask.

(6) The breath analyzing apparatus described in (4) wherein the breath collecting means is a communicating opening for connecting a breath container.

(7) The breath analyzing apparatus described in any one of (4) to (6) wherein the breath transfer means comprises a duct which connects the breath collecting means and the breath analyzing section so that the breath can flow through them.

(8) The breath analyzing apparatus described in (7) wherein the breath transfer means includes further a pump means to send breath to the breath analyzing section.

(9) The breath analyzing apparatus described in (7) or (8) wherein the breath collecting means includes both the mouthpiece or the mask and the communicating opening for connecting the breath container, and a valve which can be switched so that only one of them which is chosen depending on the case can be communicate with the breath analyzing section is provided at the duct.

(10) The breath analyzing apparatus described in any one of (3) to (9) wherein the breath analyzing section comprises a mass spectrometer.

(11) The breath analyzing apparatus described in any one of (3) to (10) used for test for hepatic cirrhosis.

The present invention also provides the above-mentioned methods and the above-mentioned apparatuses wherein the cyanides in breath is quantified by quantifying HCN which is a decomposition product thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic view showing a breath analyzing apparatus of an embodiment of the present invention.

Fig. 2 is a graph showing the relation between hepatic cirrhosis and each of an amount of isopropanol and an amount of cyanides.

Fig. 3 is a schematic view showing a breath analyzing apparatus of another embodiment of the present invention.

Fig. 4 is a schematic view showing a breath analyzing apparatus of another embodiment of the present invention.

Fig. 5 is a schematic view showing a breath analyzing apparatus of another embodiment of the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

1. A method of testing

Examples of hepatic diseases to be tested by the method of the present invention include: acute hepatitis, chronic hepatitis, fulminant hepatitis, fatty liver, and hepatic cirrhosis. Especially, the method of the present invention is suitable for test for hepatic cirrhosis.

Although it is desirable to quantify both isopropanol and cyanides in order to enable a more exact judgement, it is possible to make an exact judgement even by quantifying only one of them.

Example of cyanides may include acetonitrile.

Breath may be introduced into the apparatus directly and subjected to a quantitative analysis immediately. Alternatively, breath may be once collected in a container, then introduced into the apparatus for a quantitative-analysis after a certain time, and subjected to a quantitative analysis.

Depending on a type of quantitative-analysis conducted, the collected breath may be subjected to pretreatment such as concentration, absorption to a solution, adsorption, condensation, elimination of impurities or moisture with a filter, separation by gas chromatography, or the like, before the quantitative analysis.

The quantification of isopropanol and/or cyanides can be carried out by any method as far as the compounds can be quantified. For example, it can be carried out by mass spectrometry, spectrographic-analysis, fluorometric-analysis, gas chromatography (gas-solid chromatography, gas-liquid

chromatography), liquid chromatography, a detector tube method, a method using a semiconductor sensor, IR analysis (FT-IR or the like), or the like. Examples of the mass spectrometry may include: electronic ionization mass spectrometry, chemical ionization mass spectrometry, atmospheric pressure ionization mass spectrometry, a secondary ion mass spectrometry, fast atom bombardment ionization mass spectrometry, thermospray ionization mass spectrometry, electro spray ionization mass spectrometry, laser desorption ionization mass spectrometry, and the like. Examples of instrument to be used for analysis may include: a magnetic field single convergence type, an electric-field magnetic field double-focusing type, a quadrupole type, a 3-dimensional quadrupole type, a TOF type, and an ICR type. Moreover, GC-MS equipment, MS-MS equipment, LC-MS equipment, or the like can also be used.

Depending on the method of analysis, isopropanol and cyanides can be detected and quantified as a decomposition product or a reaction product. For example, cyanides may be quantified as a decomposition product such as HCN or the like. Isopropanol may be quantified as a decomposition product or a reaction product such as $CH_3C^*HCH_3$, CH_3C^*HOH, $(CH_3CH(OH)CH_3)_2 \cdot H_2$ or the like. Therefore, in this specification, the word "quantifying isopropanol and/or cyanides" also means quantification of isopropanol and/or cyanides performed indirectly by quantifying these decomposition products and reaction products.

In the present invention, the word "analysis" means to judge whether there is a possibility of suffering from hepatic diseases or not, using data resulted from quantification of isopropanol and/or cyanides. Judgement is conducted, for

example, by converting data of quantification analysis, such as a peak area and ionic strength, into concentration, and judging that there is no possibility of suffering from hepatic diseases when the concentration is less than a certain value, and that there is a possibility of suffering from hepatic diseases when it is more than a certain value. In this case, a certain value used as a standard of judgement (hereinafter referred to as "critical value") can be set in advance, for example, on the basis of the data obtained by measuring concentration of isopropanol and/or cyanides in the breath of six or more hepatic disease disorder patients and the same number of healthy persons in advance. As for concentration of isopropanol, a critical value can be set in the range of 0.15 ppm to 10 ppm, and can be preferably set in the range of 0.15 ppm to 1 ppm. As for concentration of hydrogen cyanide, it can be set in the range of 0.3 ppm to 10 ppm, and can be preferably set in the range of 0.5 ppm to 2 ppm. The conversion of quantitative-analysis data to concentration can be performed by a conventional method, such as a calibration curve method. Although it is desirable to perform judgement by automatic analysis using a computer, a person who conducts the test himself may also perform judgement based on the converted concentration.

Moreover, judgement can be conducted based on a relation between hepatic diseases and the quantitative-analysis data (a peak area, ionic strength, or the like) of isopropanol and/or cyanides, investigated in advance, without converting the quantitative-analysis data into concentration. Furthermore, judgement can be conducted by inputting to a data processor quantitative-analysis data of isopropanol and/or

cyanides in breath of six or more patients of the hepatic disease and healthy persons, and analyzing automatically whether the data in breath of a person to be tested is close to which of the data of the patients of a hepatic disease or the data of healthy persons.

In the above explanation, each subject is classified and judged in two groups, the one is for people without possibility of hepatic diseases and the other is for people with possibility of hepatic disease. However, it can also be classified in three or more groups gradually divided according to a possibility of a disease, a condition of a disease, or the like.

Analysis can also be performed in combination with other data, such as age, sex, a previous illness, and a factor by other test.

Examples of the factor by other test include: data of blood serum bilirubin, ZTT, TTT, ALP, CHE, GOT, GPT, γ-GTP, LDH, LAP, blood serum total protein, A/G ratio, urine bilirubin and urine urobilinogen. For example, one or more data of the above-mentioned factor can be inputted to a data processor programmed in advance for test for hepatic diseases, and the data of quantification of isopropanol and/or cyanides can be analyzed automatically together with these data.

The method of judgement is not limited to the above-mentioned method. Moreover, a critical value can also be suitably chosen according to the standard of classification adopted, various factors by other tests as mentioned above, the precision of hepatic diseases screening to be intended, or the like. It was found out by the present invention that the concentration of isopropanol and cyanides in breath of a

healthy person significantly differs from those of a hepatic disease patient. Thus, a judging method suitable for the test actually conducted can be adopted by collecting the data of quantification of isopropanol and a cyanide in breath of, for example, six healthy persons and the same number of hepatic disease patients.

2. Breath Analyzing apparatus

As to the apparatus of the present invention, the breath collecting section is a section for collecting the breath to be analyzed, introducing it into the apparatus, and leading it to the breath analyzing section. It preferably consists of a breath collecting means for collecting breath, and a breath transfer means for transporting the collected breath to the breath analyzing section.

The breath collecting means may be, for example, a breath blowing-in opening such as a mouthpiece for collecting breath directly, a mask in the form which covers a mouth or both a nose and a mouth, a communicating opening to which a breath container is connected, or the like.

When the test is performed by introducing breath into the apparatus directly, the above-mentioned blowing-in opening is used. When the test is performed by collecting breath in a breath container and introducing it into the apparatus for quantification after a certain time, the above-mentioned communicating opening is used.

The breath container is a container for collecting the breath to be analyzed. Examples thereof include: a glassware like a vacuum bottle, a breath collecting bag made of a synthetic resin, for example, the products made of elasticity

vinyl chloride, vinyl tetrafluoride, tetraethylene fluoride, and a polyethylene phthalate and the like.

The above-mentioned mouthpiece and mask may have a structure which can collect breath efficiently without leaking it outside. Furthermore, it is desirable to provide a means for preventing blown-in breath from leaking to the open air, for example, a valve.

The breath transfer means may include, for example, a duct which connects the breath collecting means and the breath analyzing section so that breath may pass through, a valve provided in the duct, a pump for sending or introducing breath to the breath analyzing section compulsorily.

In order to suppress adsorption of the substance to be tested in breath to the duct, the inside of the duct is desirably subjected to electropolishing. Furthermore, for the same purpose as the above, the apparatus of the present invention is equipped with a heating means for heating a breath collecting section, especially the duct and the valve, to a constant temperature.

Furthermore, the breath transfer means may include a breath amount control mechanism. It is desirable to have a structure which makes it possible to send a certain amount of the collected breath to the breath analyzing section.

Moreover, in the breath collecting section, there may be provided a pretreatment means for subjecting the breath to pretreatment such as concentration, absorption to a solution, adsorption, condensation, elimination of impurities or moisture with a filter, separation by gas chromatography or the like.

In the present invention, the breath analyzing section

is a section wherein isopropanol and/or cyanides in breath is quantified, and this section includes a quantitative-analysis instrument which can quantify these substances.

Specific examples of the quantitative-analysis instrument may include: a mass spectrometer, a spectrographic-analysis meter, a fluorometric-analysis meter, gas-chromatograph equipment (gas-solid chromatograph equipment, gas-liquid chromatograph equipment), liquid chromatograph equipment, an indicator tube, a semiconductor sensor, IR analyzer (for example, FT-IR), an ion-electrode concentration measuring apparatus, a photoelectric photometer, the colorimeter, and the like. Preferable examples of the mass spectrometer include: an electronic ionization mass spectrometer, a chemical ionization mass spectrometer, an atmospheric-pressure-ionization-mass-spectrometry meter, a secondary ion mass spectrometer, a fast-atom-bombardment ionization mass spectrometer, a thermospray ionization mass spectrometer, an electro spray ionization mass spectrometer, a laser desorption ionization mass spectrometer, or the like. The separation of ions may be for example, a magnetic field single convergence type, an electric-field magnetic field double-focusing type, a quadrupole type, a 3-dimensional quadrupole type, a TOF type, and an ICR type. Moreover, GC-MS equipment, MS-MS equipment, LC-MS equipment, or the like can be used.

In the present invention, the "data-processing section" is a section where judgment as for the hepatic diseases is carried out by analyzing the result obtained by the breath analyzing section. If desired, there are also performed in the section calculation of concentration of isopropanol and/or

cyanides, judgment of a possibility of hepatic diseases, i.e., the judgment as for hepatic disease explained in detail in the above item as for the method of testing, and/or display of the results or the like.

As described above, a computer program may perform all data processing for a judgment automatically in the data-processing section. Alternatively, only calculation of concentration of isopropanol and/or cyanides and display of concentration may be performed in the data-processing section, and an inspector may perform a judgment.

The data-processing section may have a database comprising quantified values of isopropanol and cyanides in the breath of two or more hepatic disease patients measured in advance, and quantified values of isopropanol and cyanides in a healthy person's breath. In that case, a test for hepatic diseases can be conducted by comparing the above-mentioned measured value with the database.

Although the apparatus of the present invention may be mainly used for the purpose of screening of hepatic diseases in a medical checkup or the like, it can also be used for assistance of a diagnosis in a medical institution. Moreover, if remote places, such as a doctorless village and a doctor of a hospital in a city using a communication line are connected each other to transmit analysis results or the like, monitoring of conditions of a person under a long-term medical-treatment and inspection for a teletherapy and the like can also be enabled.

The present invention will be explained further in detail by the following examples. However, they do not limit

the scope of the present invention. The skilled in the art may carry out various improvement and modification based on the description of the present invention and information known in the art. In all drawings showing the examples, the same numerals are used for the parts having the same function respectively, and same explanation is not repeated for those parts.

Example 1

Fig.1 shows a schematic view of the breath analyzing apparatus of this example.

The apparatus for testing for hepatic cirrhosis of this Example, that is a breath analyzing apparatus 1a consists of a breath collecting section 28, a breath analyzing section 29, and a data-processing section 30 as shown in Fig. 1. In the breath collecting section 28, a mouthpiece 2 and a breath collecting bag 6 are connected to a three-way valve 5 by an duct 52. Switch valves 3 and 4 provided in the valve 5 is used for switching between the case where breath is directly introduced from the mouthpiece and the case where breath is indirectly introduced after once collected in the breath collecting bag 6. The above-mentioned valve 5 is connected to a breath introduction opening of the breath analyzing section 29 via a flow controller 7, and controls a flow of the breath introduced directly or indirectly to a certain value, and introduces it into the breath analyzing section 29.

A duct of which inner surface is subjected to electropolishing is used as the duct constituting the breath collecting section 28, and the duct and the valve 5 are heated to a constant temperature by a heater 8. Thereby, adsorption

of the substance in breath to be analyzed to the duct is suppressed.

Atmospheric pressure ionization mass spectrometry meter (hereinafter referred to as APIMS) which can conduct trace analysis is used as the analyzer of the breath analyzing section 29, and thereby the substances to be analyzed in breath can be analyzed with very high sensitivity. The cylinder of the Ar + H_2 (1 %) mixed gas 9 as primary-ion generation gas is connected to a first ionization chamber 15 of the APIMS through a reducing valve 10 and a flow controller 11, and primary ion is generated by corona discharge by high pressure applied to an electric discharge needle 16. Moreover, the breath collecting section 28 and a diaphram pump 13 via a tension controller 12 are connected to a second ionization chamber 17. When the inner pressure of the second ionization-chamber 17 is kept at 0.85 Pa so that the breath gas may be sucked from the breath collecting section 28, and the primary ions generated by the first ionization chamber 15 and neutral molecules of the substances to be analyzed are collided, a ion molecular reaction is caused and the substances to be analyzed are ionized. A differential-pumping section 18 is a section which connects the 2nd ionization chamber 17 and the breath analyzing section 23, which is maintained at the low vacuum by an evacuation system 20. The breath analyzing section 23 is maintained at high vacuum by an evacuation system 21. A quadrupole mass spectrometer 22 is provided therein, and the ions introduced into the breath analyzing section 23 through a thin opening of a slit 19 is separated by mass-spectroscopy, and is converted into an electrical signal. A signal amplifier 24 is connected to the breath analyzing section 23,

and amplifies the electrical signal converted by the breath analyzing section 23, and transmits it to a data-processing section 30.

The data-processing section 30 consists of a computer 25, a database 26 and a display 27, and calculates both or one of concentrations of isopropanol and cyanides which are the substances in breath to be analyzed from the signals transmitted from the signal amplifier 24, and compare it with a database 26 created in advance using concentration of isopropanol or cyanides in breath of a hepatic cirrhosis patient group and of a healthy person group (those were accepted to be normal at medical checkup). Whether it suffers from hepatic cirrhosis or is normal is determined by judging to which group it is close, then the result is displayed on the display 27.

Operation of this Example will be explained below. When breath is directly introduced, it is introduced from the mouthpiece 2, and the switch valve 3 at this time is set as "open", and the switch valve 4 is set as "close". When breath is indirectly introduced, breath is once collected in the breath collecting bag 6, and then the breath collecting bag 6 is connected to the valve 5. At that time, the switch valve 3 is set as "close", and the switch valve 4 is set as "open", and the breath is introduced. Flow control of the introduced breath is carried out by the flow controller 7, and is then introduced into the second ionization chamber 17 of APIMS. On the other hand, the Ar + H_2 (1 %) mixed gas 9 as the primary-ion generation gas is controlled at a certain pressure by a reducing valve 10, and flow control thereof is carried out by the flow controller 11, and is introduced into the first

ionization chamber 15 of APIMS. The introduced Ar + H_2 (1 %) mixed gas 9 produces a corona discharge by the high voltage applied to the electric discharge needle 16, and, as a result, primary ions are generated. The generated primary ions are introduced into the second ionization chamber 17 and mixed with the breath introduced from the breath collecting section 28. As a result of being mixed, breath is collided with primary ions, to cause an ion-molecular reaction, and the substances to be analyzed in breath are ionized. The ionized substances pass through the differential-pumping section 18, are introduced into the breath analyzing section 23, are separated by the quadrupole mass spectrometer 22, and then are converted to electrical signals and are output. The converted electrical signals are amplified by the signal amplifier 24, then transmitted to the data-processing section 30. Both or one of concentrations of isopropanol and cyanides are calculted from the transmitted signals, and compared with the database 26 created in advance using an isopropanol or cyanides concentration in breath of a hepatic cirrhosis patient group and of a healthy person group (those were accepted to be normal at medical checkup). Whether it is hepatic cirrhosis or normal is determined by judging to which grope it is close.

The breath of 20 healthy persons and 20 hepatic cirrhosis patients were analyzed using the apparatus of Fig. 1. The results of mass analysis are shown in Fig. 2. Comparison of concentration of cyanides in hepatic cirrhosis patients to healthy persons is shown in Fig. 2 (A). Comparison of concentration of isopropanol in hepatic cirrhosis patients to healthy persons is shown in Fig. 2 (B). It is clear from the

graph that concentration of cyanide and isopropanol of a hepatic cirrhosis patient's group is 4 to 10 times as a healthy person's group. According to this Example, a simple test for hepatic cirrhosis can be conducted by analyzing cyanide and isopropanol in breath using the breath analyzing apparatus equipped with APIMS.

Example 2

Fig.3 shows a schematic view of the breath analyzing apparatus 1b of this example. In this example, an ion trap mass spectrometer 34 is used in a breath analyzing section. Since the ion trap mass spectrometer 34 is an analyzer which makes microanalysis possible, as in the case of APIMS, the substance to be analyzed in breath can be analyzed with high sensitivity. The ion trap mass spectrometer 34 consists of an ionization section 31 and a high-vacuum section 32. There is provided an ionization means by electric discharge or the like in the ionization section 31, which ionizes the breath introduced from the breath collecting section 28. The high-vacuum section 32 is the section maintained at high vacuum by an evacuation system 36. An ion trap electrode 33 and a detector 35 are provided therein. Ions generated in the ionization section 31 are subjected to trap concentration, which are then detected by the detector 35. Then, they are converted to an electrical signal and transmitted.

As described above, according to this Example, a simple test for hepatic cirrhosis can be conducted by analyzing cyanides and isopropanol in breath using the breath analyzing apparatus with the ion trap mass spectrometer 34.

Example 3

Fig.4 shows a schematic view of the breath analyzing

apparatus 1c of this example. In this example, a gas-chromatograph mass spectrometer 42 is used in a breath analyzing section. Since both a qualitative analysis and a quantitative analysis can be conducted simultaneously by the gas-chromatograph mass spectrometer 42, breath analysis can be performed without identifying a peak in advance. The gas-chromatograph mass spectrometer 42 consists of a carrier gas introducing section, a column 37, an interface 38, and a mass spectrometer 39. In the carrier gas introducing section, the carrier gas cylinder 43 is connected with the column 37 via the reducing valve 44 and the flow controller 45. Thereby, the carrier gas can be supplied to a column 37 at a certain pressure and at a certain flow. In the column 37, the substances are separated by difference in chemisorption of the substance. The interface 38 connects the mass spectrometer 39 to the column 37, and controls a gas flow, measurement timing or the like. The mass spectrometer 39 is maintained at a high vacuum by the evacuation system 40, and the separated ions are detected by the detector 41, converted to an electrical signal, and then transmitted.

Operation of this Example will be explained below. The carrier gas maintained at a certain pressure by the reducing valve 44 and maintained at a certain flow by the flow controller 45 is introduced into the column 37 with the breath introduced by the breath collecting section 28. The substance to be analyzed in the introduced breath is introduced into the mass spectrometer 39 through the interface 38, after being separated by feature of the substance. In the mass spectrometer 39, they are ionized and separated, then detected by the signal detector 41, and converted to electrical signal

and transmitted. According to this Example, a simple test for hepatic cirrhosis can be conducted by analyzing cyanides and isopropanol in breath using the breath analyzing apparatus with the gas-chromatograph mass spectrometer 42.

INDUSTRIAL APPLICABILITY

According to a test method of the hepatic diseases of the present invention and the breath analyzing apparatus therefor, a test for hepatic diseases can be conducted without requiring an engineer having special technology, without giving a subject pain, and the results can be obtained immediately.

Moreover, a still more exact judgment of hepatic diseases can be achieved by combining with other test results.

Therefore, a simple, exact, quick test for hepatic diseases can be conducted not only at medical institutions, such as a hospital, but also at a medical checkup center or a health center.

Furthermore, the apparatus of the present invention makes a teletherapy such as monitoring of a person recuperated at home in a remote place or the like possible.